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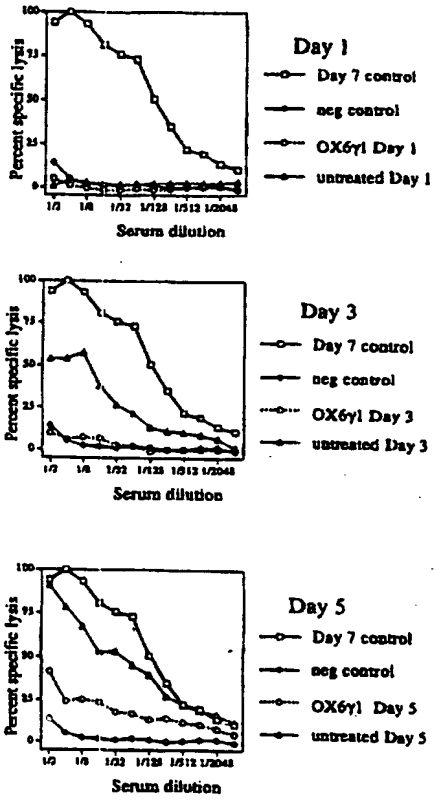
(54) Title: ANTI-CLASS II MHC BINDING AGENTS FOR USE IN XENOTRANSPLANTATION

(57) Abstract

Pharmaceutical products are described which suppress or eliminate the rejection of xenografts. Each product is based on the use of a Class II major histocompatibility (MHC) binding agent, such as an anti-MHCII antibody to inhibit T-cell independent B-cell function during the delayed acute rejection phase of the xenograft. The combined use of the Class II MHC binding agent with an immunosuppressant capable of suppressing T-cell activation, such as a cyclosporin, and optionally an inhibitor of B-cell function such as cyclophosphamide achieve prolongation of graft survival beyond that which can be achieved by the use of each agent alone.

Anti-hamster antibody titre from OX6γ1 treated and untreated xenografts.



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ANTI-CLASS II MHC BINDING AGENTS FOR USE IN XENOTRANSPLANTATION

This invention relates to pharmaceutical products for the suppression or elimination of the rejection of xenografts and to the manufacture of such products.

One of the major factors influencing the number of solid organ allografts in human transplantation is the availability of suitable human donor organs. Once organs are available, modern surgical techniques and immunosuppressive therapy, although by no means ideal in terms of patient compliance, can then usually prolong survival. The shortage of suitable human organs however means that in many cases there is a long wait for a transplant. This wait may be fatal, particularly for patients awaiting a heart or liver transplant.

Transplantation of organs from non-human species (xenografting) would avoid the problem of human organ availability. Xenografting currently does not succeed however because of pre-existing anti-species antibodies in the human host which mediate hyperacute rejection. The rejection process is thought to be due in large part to a T-cell independent B cell effect, which in discordant grafting, (a transplant between distantly related species, e.g. pig to human) can result in a vigorous, complement-mediated destruction of the graft within minutes. With a concordant graft (a transplant between closely related species, e.g. baboon to human) the hyperacute response (sometimes called delayed acute rejection) can be less vigorous and occurs within a few days but is again due to T-cell independent B cell effects.

The B-cell effect has been illustrated in rats in which hamster-to-rat (concordant) heterotopic heart transplants reject in around 3 days. This is not prolonged by normal T-cell immunosuppressive treatment using cyclosporin A with doses which would normally prolong allograft survival indefinitely. However, if recipients are treated with cobra venom factor or cyclophosphamide to inhibit complement activity or lymphocyte proliferation this initial rejection phase can be overcome. T-cell

immunosuppression can then be used [van den Bogaerde, J *et al*, Transplant Proc. 24, 513-514 (1992); Hasan, R. I. R. *et al ibid*, 24, 517-518 (1992)].

Recent progress using animals (e.g. pig) transgenic for human complement regulatory protein (e.g. DAF) has shown that many of the complement mediated effects can be overcome, for example in a pig to cynomolgus monkey xenograft. However, large doses of cyclophosphamide are still needed to suppress the T-cell independent B cell response. In a clinical situation this would not be acceptable due to the marked toxicity of cyclophosphamide. Ideally an agent which could reduce B cell responses during xenotransplantation, at least during the early phase and to a degree where an immunosuppressant such as cyclophosphamide either need not be administered or may be used at clinically acceptable doses, would be useful.

We have now surprisingly found that an antibody to a rat Class II major histocompatibility complex protein can inhibit B-cell function *in vivo* and is capable of prolonging the time to rejection of a xenograft in a hamster-to-rat xenograft model. Unexpectedly, when the antibody is used in conjunction with the immunosuppressant cyclosporin A in the same model the survival of the xenograft is markedly prolonged beyond the times when rejection occurs in the presence of one or other of the antibody or cyclosporin A alone. This has allowed us to develop a general means to suppress or eliminate rejection of a xenogeneic transplant which in particular avoids the use of potentially toxic doses of cyclophosphamide and similarly acting compounds.

Thus according to one aspect of the invention we provide a pharmaceutical product comprising a Class II major histocompatibility complex protein binding agent for use to suppress or eliminate the rejection of a xenograft in a host.

In the specification hereinafter the abbreviation "MHC" is used to represent the term "major histocompatibility complex".

The term "xenograft" is used in conventional fashion to mean a graft derived from a donor species unrelated to the host species. The donor and host species may be for example any mammalian species and may be concordant, or closely related, e.g. chimpanzee or baboon donors and a human host; or discordant, or distantly related, e.g. a swine donor and a human host. The graft may be any organ or tissue (including isolated cells), and may be in particular a solid organ such as a heart, liver or kidney, or a tissue such as pancreatic tissue, e.g. pancreatic β-islet cells.

The product according to the invention can be used either alone, or advantageously in conjunction with at least one other active ingredient, in particular with a further, different immunosuppressant agent. The invention thus extends to a pharmaceutical product comprising a Class II MHC protein binding agent and at least one other, different immunosuppressant for simultaneous combined, simultaneous separate or sequential use to suppress or eliminate the rejection of a xenograft in a host.

One such pharmaceutical product may take the form of a pharmaceutical composition in which the Class II MHC protein binding agent and the other immunosuppressant(s) are formulated in admixture, optionally together with a pharmaceutically acceptable excipient, diluent, or carrier and the invention extends to such compositions. Alternatively, the product according to the invention may take the form of a separately formulated Class II MHC protein binding agent and separately formulated other immunosuppressant(s) optionally presented together for simultaneous or sequential use.

In the products according to the invention the Class II MHC protein binding agent may be any agent capable of binding to Class II MHC protein in the host and/or donor so that any host T-cell independent B-cell response is suppressed or eliminated. One particular class of such agents contains antibodies which recognise the Class II MHC protein and in so doing inhibit any T-cell independent B-cell response. The invention thus especially extends to the above-mentioned pharmaceutical products and to their manufacture and use described hereinafter in which the Class II

MHC protein binding agent is an antibody to a Class II MHC protein. Except where otherwise indicated, an antibody of this type is hereinafter referred to as an anti-MHC II antibody.

Anti-MHC II antibodies are well-known in the art to interfere with T-cell activation [see for example Wraith, D. C. *et al*, Cell, 57, 709-715 (1989)]. On the basis of this they have been suggested previously for use in the treatment of immunological diseases, including transplant related conditions such as graft versus host disease and allograft rejection where it is important to suppress inappropriate T-cell activation (see for example International Patent Specification No. WO 94/29451). In the present invention, selection of the anti-MHC II antibody is based on its ability to inhibit T-cell independent B-cell function, and use is made of this during the delayed acute rejection phase of the xenograft. Once past the delayed acute rejection phase the continued presence of the antibody may also be beneficial in helping to suppress any T-cell mediated rejection of the graft.

The anti-MHC II antibody for use in the product according to the invention may be a whole antibody or an antigen binding fragment thereof, for example a Fab fragment or, especially, a bivalent fragment, such as a $F(ab')_2$ fragment. The antibody may be of animal, for example mammalian origin and may be for example of murine, rat or human origin. It may be polyspecific, but is preferably monospecific for a MHC II protein, especially a human MHC II protein. The antibody will in particular be one which is capable of inhibiting a T-cell independent B-cell response. It may be a polyclonal antibody or, preferably, a monoclonal antibody. Where desired, it may be a labelled antibody, the label being for example a reporter or effector group.

A number of anti-MHC II antibodies for use in the invention can be obtained from either freely or commercially available hybridoma cell lines, for example the cell lines ATCC accession No. HB55 and ATCC Accession No. HB151 [American Type Culture Collection, Rockville, Maryland, USA] producing the antibodies L243 and SFR3-DR5 respectively. Alternatively the antibody may be obtained using conventional immunisation and/or recombinant DNA techniques.

Thus, for example polyclonal antibodies may be obtained from the sera of animals immunised with a Class II MHC protein immunogen. Well known methods may be used to obtain the immunogen either from readily available cell sources or gene and/or protein sequence data [see for example Culley, D *et al*, Cell. Immunol. 149, 279-290 (1993); Andersson, G *et al*, J. Biol. Chem. 262, 8748-8758 (1987); Servenius, B *et al*, *ibid* 262, 8759-8766 (1987), Jonsson, A-K *et al*, *ibid* 262, 8767-8777 (1987)]. Any suitable host, for example BALB/c mice where it is desired to obtain a mouse polyclonal antibody, may be injected with the immunogen, the serum collected and the antibody recovered therefrom. Monoclonal antibodies may be obtained from hybridomas derived from the spleen cells of an animal immunised as just discussed and fused to an appropriate "immortal" B-tumour cell. In each instance, the antibody may be recovered from either the serum or the hybridoma by making use of standard selection and subsequent purification and or concentration techniques, for example by chromatography, using for example Protein A or by other affinity chromatography. Selection of the antibody may be by any conventional selection means, for example by use of one or more cell based assay systems utilising appropriate indicator cell lines, for example as described in International Patent Specification No. WO 94/29451. In particular, part of the selection process will be based on the ability of the antibody to inhibit T-cell independent B-cell function.

Once a cell line, for example a hybridoma, expressing an antibody for use in the invention has been obtained it is possible to clone therefrom the cDNA and to identify the variable region genes encoding the desired antibody, including the sequences encoding the CDRs. From here, other recombinant antibodies for use in to the invention may be obtained by preparing one or more replicable expression vectors containing at least the DNA sequence encoding the variable domain of the antibody heavy or light chain and optionally other DNA sequences encoding remaining portions of the heavy and/or light chains as desired, and transforming an appropriate cell line, e.g. a non-producing myeloma cell line, such as a mouse NSO line, in which production of the antibody will occur. In order to obtain efficient transcription and translation, the DNA sequence in each

vector should include appropriate regulatory sequences, particularly a promoter and leader sequence operably linked to the variable domain sequence. Particular methods for producing antibodies in this way are generally well known and routinely used. For example, basic molecular biology procedures are described by Maniatis *et al* [Molecular Cloning, Cold Spring Harbor Laboratory, New York, 1989]; DNA sequencing can be performed as described in Sanger *et al* [PNAS 74, 5463, (1977)] and the Amersham International plc sequencing handbook; and site directed mutagenesis can be carried out according to the method of Kramer *et al* [Nucl. Acids Res. 12, 9441, (1984)] and the Anglian Biotechnology Ltd handbook. Additionally, there are numerous publications, including patent specifications, detailing techniques suitable for the preparation of antibodies by manipulation of DNA, creation of expression vectors and transformation of appropriate cells, for example as reviewed by Mountain A and Adair, J R in Biotechnology and Genetic Engineering Reviews [ed. Tombs, M P, 10, Chapter 1, 1992, Intercept, Andover, UK] and in International Patent Specification No. WO 91/09967.

In the above aspects of the invention, and in those described hereinafter, the xenograft may be from a non-human species and the host is a human host. In these circumstances, when the Class II MHC protein binding agent is an anti-MHC II antibody, the antibody is preferably an anti-human MHC II antibody. Particular examples of such antibodies are the L243 and SFR3-DR5 antibodies and fragments thereof described above. In one particular preference the antibody is a recombinant version of one of these, particularly the L243 derived recombinant antibodies described in International Patent Specification No. WO 94/29451 and especially the antibody L243-gH/L243-gL1 described therein.

As explained above, and described in the Example hereinafter, it is advantageous to use the Class II MHC protein binding agent, particularly the anti MHC II antibody, in conjunction with at least one other immunosuppressant. One particular class of immunosuppressant for such a use according to the invention is a class containing agents capable of suppressing T-cell activation. Numerous examples of such agents are well known in the art and some are in routine use in the clinic, for example

in the treatment of allograft rejection [see for example Morris, R. E. Transplantation Society Bulletin 1, 15-20 (1993)]. Particular examples include: (1) compounds which inhibit cytokine synthesis, for example the cyclosporins, e.g. cyclosporin A and analogues or derivatives thereof such as cyclosporin G or the hydroxyethyl derivative of D-serine cyclosporin A [see for example Burdmann, E. A *et al*, J. ASN 3, 841 (1992); and Hiestand, P. C. *et al*, Transplant. Proc. 25, 691-692 (1993)] or tacrolimus (FK506) or analogues or derivatives thereof [see for example Klintmalm, G.B. Transplant. Proc. 28, 974-976 (1996)]; (2) compounds which inhibit cytokine action, for example macrolide immunosuppressants such as rapamycin and analogues or derivatives thereof, or isoxazoles such as leflunomide and analogues or derivatives thereof; [see for example Thompson, A.W. Immunol. Lett. 29, 105-112 (1991); and Bartlett, R. R. *et al*, Agents Actions 32, 10-21 (1991)] (3) inhibitors of DNA synthesis, such as mizoribine, mycophenolic acid or brequinar; [see for example Cramer, D.V. *et al* Transplantation 53, 303 (1992); and Gray, D. W. R. Lancet 346, 390 (1995)] or (4) inhibitors of cell maturation such as deoxy-spergualin [see for example Ameniya, H *et al*, Transplant Proc. 23, 1087 (1991)].

If desired more than one immunosuppressant may be used in conjunction with the Class II MHC protein binding agent in the products according to the invention. In addition the products may also contain other active ingredients, for example inhibitors of B-cell function such as cyclophosphamide. In each instance, as explained previously, the presence of the Class II MHC protein binding agent allows, where desired, the other active ingredients to be used at doses where any unacceptable side-effects are reduced or avoided.

Formulation of the Class II MHC protein binding agent and any other active ingredient for use in a product according to the invention may be carried out using conventional procedures. Each active ingredient may take any suitable form for administration to the host, for example a form for oral, parenteral or rectal administration.

Where the active ingredient is for parenteral administration, for example for intravenous, intramuscular or subcutaneous injection or infusion, it may

be presented in unit dosage form, e.g. in glass ampoule or multi dose containers, e.g. glass vials. It may be formulated as a suspension, solution or emulsion in an oily or aqueous vehicle optionally containing formulatory agents such as suspending, stabilising, preserving and/or dispersing agents. Alternatively the active ingredient may be in a dry form, e.g. a powder for reconstitution before use with an appropriate sterile liquid, e.g. sterile pyrogen-free water.

For oral administration, the active ingredient may take the form of, for example, tablets, lozenges or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g. pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g. lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g. magnesium stearate, talc or silica); disintegrants (e.g. potato starch or sodium glycollate); or wetting agents (e.g. sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents, emulsifying agents, non-aqueous vehicles and preservatives. The preparations may also contain buffer salts, flavouring, colouring and sweetening agents as appropriate.

Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For rectal administration the active ingredient may be formulated with a binding and/or lubricating agent, for example with a polymeric glycol, a gelatin, cocoa-butter or other vegetable wax or fat.

The active ingredient(s) may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing each active ingredient. The pack or dispensing device may be accompanied by instructions for administration.

Where in the product according to the invention the Class II MHC protein binding agent is an anti-MHC II antibody this is likely to be unsuitable for oral administration and it is preferably used in a formulation for parenteral administration using for example one of the approaches described above.

In one of the aspects of the invention described above the product comprises a Class II MHC protein binding agent and at least one other immunosuppressant in admixture and according to a further aspect of the invention we therefore provide the use of a Class II MHC protein binding agent and at least one other, different immunosuppressant in the manufacture of a pharmaceutical product for use to suppress or eliminate the rejection of a xenograft in a host. Thus for example the Class II MHC protein binding agent and the immunosuppressant(s) may be mixed together and other ingredients, e.g. a pharmaceutically acceptable excipient, diluent or carrier, also mixed in as required, to yield for example a product formulated for oral, parenteral or rectal administration as described previously.

The products according to the invention will contain active ingredients at a therapeutically effective dose and in a further aspect of the invention we provide a method of suppressing or eliminating the rejection of a xenograft in a host, the method comprising administering to the host a pharmaceutical product comprising an effective amount of a Class II MHC protein binding agent. In this aspect of the invention the pharmaceutical product may also contain an effective amount of one or more other, different immunosuppressants as described above.

Administration of the products according to the invention to the host generally will be before, during and/or after the transplant operation. In particular, however, to avoid the hyperacute rejection associated with the T-cell independent B-cell response it will be necessary to ensure that at least the Class II MHC protein binding agent is present in sufficient quantity in the host at the beginning of the transplant procedure. In practice this may mean administering at least the Class II MHC protein binding agent to the host prior to the transplant procedure for one or more days. In addition, it may be advantageous to ensure that the organ or

tissue to be transplanted is contacted with the Class II MHC protein binding agent prior to the transplant procedure, for example by bathing the isolated organ or tissue in a sterile solution containing the agent. The administration of the Class II MHC protein binding agent may be continued during or after the transplant operation for as long as the xenograft is at risk from an hyperacute rejection. The administration may then be discontinued as desired and reintroduced as necessary. Where the invention utilises other immunosuppressants these may generally be administered at the time of the transplant and thereafter on a continuous basis for as long as it is desired to suppress or eliminate any T-cell mediated rejection.

The doses at which the Class II MHC protein binding agent and other immunosuppressant(s) will be administered will depend for example on the nature of the xenograft and other factors such as the age and condition of the host and the route of administration. In general the active ingredients will be used at doses generally recognised to be effective for the class of compound involved. Thus, for example where the Class II MHC protein binding agent is an anti-MHC II antibody this may be used at a dose between 0.1 - 100mg/Kg, e.g. 0.1 - 50mg/kg, preferably 0.1 - 10mg/kg body weight per single dose, depending on the nature, e.g. avidity of the antibody. Other immunosuppressants, for example the T-cell activation inhibitors described above, may be used at doses within the range 0.01 - 50mg/kg, preferably 0.05 - 20 mg/kg body weight per single dose. The doses may be administered singly one or more times a day or continuously during a day up to a maximum effective or tolerated total dose. Where appropriate initial daily doses may be varied once the hyperacute rejection phase has been passed, for example to reduce the frequency and/or quantity of dose.

EXAMPLES

The following Examples illustrate the invention and demonstrate the effect of an anti-rat MHC II mouse monoclonal antibody (OX-6) in a hamster-to-rat xenograft model. The model used in each Example was as follows:

Graft Survival Studies

Hearts from male hamsters were transplanted into the abdomens of rat (DA strain, RT1^{avl}) recipients and viability of each graft was assessed by external palpation on a grade of 0-4 (with 4 indicating a normally beating graft and 0 indicating rejection). The anti class II Mab used was OX-6 (anti-rat RT1-B monoclonal antibody; European Collection of Animal Cell Cultures, ECACC No. 84112007).

The hamster-to-rat xenograft provides a recognised model for xenogeneic grafting between other species and the results obtained would be expected to be found, for example, in non-human-to-human xenografts, for example swine-to-human.

Example 1

Effect of OX6 IgG alone or with Cyclosporin A (CsA)

DA rat recipients were treated as shown in Table 1

TABLE 1

Treatment	Time to rejection (days)
Saline only	3, 3, 3, 3, 3, 3
OX6 (40mg/kg IP (day -1, 0, 1)	5, 5, 5, 5, 5, 5
CsA (30mg/kg PO daily from day 0)	3, 3, 3, 3
OX6 (40mg/kg day -1, 0, +1) and CsA (30mg/kg day 0-7 then 20mg/kg every other day)	9, 65, 65+, 73, 100+, 100+

"+" denotes that the graft had not rejected at the time the animal was examined.

These data indicate that OX-6 IgG alone can prolong the time to rejection of a xenogeneic graft by 2 days and, when in combination with an immunosuppressive dose of cyclosporin A, can markedly increase the survival time beyond that of either agent alone.

Example 2

Effect of OX-6 F(ab')$_2$ alone or with cyclosporin A (CsA)

DA rat recipients were treated as shown in Table 2.

TABLE 2

Treatment	Time to rejection (days)
Saline only	3, 3, 3, 3, 3, 3
OX6 F(ab')$_2$ (80mg/kg IP days -1, 0, +1)	4, 4, 4, 5, 5, 5
OX6 F(ab')$_2$ (80mg/kg IP, days -1, 0, +1) and CsA (30mg/kg days 0-7 then 20mg/kg every other day PO)	11, 26, 27, 41+ 42+, 67, 67+, 95+, 95+

"+" denotes that the graft had not rejected at the time the animal was examined.

These data indicate that OX-6 F(ab')$_2$ alone can prolong the time to rejection of a xenogeneic graft by 2 days and, when in combination with an immunosuppressive dose of cyclosporin A, can markedly increase the survival time beyond that of either agent alone.

Example 3

Effect of OX-6 IgG alone or with mycophenolic acid (MMF)

DA rat recipients were treated as shown in Table 3.

TABLE 3

Treatment	Time to rejection (days)
Saline only	3, 3, 3, 3, 3, 3
OX6 IgG (40mg/kg IP days -1, 0, +1)	5, 5, 5, 5, 5, 5
MMF (40mg/kg PO daily)	4, 5, 5, 5, 5, 5
OX6 IgG (40mg/kg IP day -1, 0, +1) and MMF (40mg/kg daily)	8, 8, 8, 8, 9, 9

These data indicate that OX-6 IgG in combination with an immunosuppressive dose of mycophenolic acid can increase graft survival time beyond that of either agent alone.

Example 4

Effect of OX6 IgG alone or with tacrolimus (FK506

DA rat recipients were treated as shown in Table 4.

TABLE 4

Treatment	Time to rejection (days)
Saline only	3, 3, 3, 3, 3, 3
OX6 IgG (40mg/kg IP days -1, 0, +1)	5, 5, 5, 5, 5, 5
FK506 (1mg/kg IP daily)	3, 3, 3, 4, 4
OX6 IgG (40mg/kg IP, days -1, 0, +1), and FK506 (1mg/kg IP daily)	6, 6, 6, 6, 7, 13+

"+" denotes that the graft had not rejected at the time the animal was examined.

These data indicate that OX-6 IgG in combination with an immunosuppressive dose of FK506 can increase graft survival time beyond that of either agent alone.

Example 5

Inhibition of B-cell function

Materials and Methods

Anti-hamster lytic antibody assay

Transplanted DA rats (see 'Graft Survival Studies' above) were given either saline, OX68[1] or $(Fab')_2$OX6 intraperitoneally at 40mg/kg and 80mg/kg, respectively, on days -1 and 0. Groups of rats were bled following surgery on days 1, 3, 5 and 7. The sera from these samples were assayed for anti-hamster titre as follows. In a 96 well round bottomed microtitre plate, 25µl of a 1% hamster erythrocyte suspension was added to 25µl of test serum that had been serially diluted in complement fixation diluent (CFD). To this 25µl of a 1:7 dilution of baby rabbit complement (Serotec, Oxford, UK) was added and incubated for 1.5h at 37°C. CFD (150µl) was added and the plate centrifuged at 150g for 4 minutes before 150µl of supernatant was transferred into an ELISA plate and the Absorbance read at 415nm wavelength. Positive and negative controls were distilled water or CFD, respectively.

Figures 1 and 2 illustrate the results obtained in one experiment. The data indicates that OX6 IgG8[1] or $F(ab')_2$ can inhibit xenogeneic antibody production in DA rat recipients. This demonstrates that OX6 can inhibit B cell function *in vivo*.

CLAIMS

1. A pharmaceutical product comprising a Class II major histocompatibility complex protein binding agent for use to suppress or eliminate the rejection of a xenograft in a host.

2. A pharmaceutical product according to Claim 1 comprising a Class II major histocompatibility complex protein binding agent and at least one other, different immunosuppressant for simultaneous combined, simultaneous separate or sequential use to suppress or eliminate the rejection of a xenograft in a host.

3. A pharmaceutical product according to Claim 1 or Claim 2 wherein the Class II binding agent is capable of suppressing or eliminating a host T-cell independent B-cell response.

4. A pharmaceutical product according to Claim 3 wherein the Class II binding agent is an antibody.

5. A pharmaceutical product according to any one of Claims 2 to 4 wherein the other immunosuppressant is capable of suppressing T-cell activation.

6. A pharmaceutical product according to Claim 5 wherein the immunosuppressant is selected from (1) compounds which inhibit cytokine synthesis; (2) compounds which inhibit cytokine action; (3) inhibitors of DNA synthesis; or (4) inhibitors of cell maturation.

7. A pharmaceutical product according to Claim 6 wherein the immunosuppressant is selected from a cyclosporin or tacrolimus or analogues or derivatives thereof.

8. A pharmaceutical product according to any one of Claims 1 to 7 additionally containing an inhibitor of B-cell function.

9. A pharmaceutical composition comprising a Class II major histocompatibility complex binding agent and at least one other, different immunosuppressant in admixture, optionally together with a pharmaceutically acceptable excipient, diluent, or carrier.

10. A method of suppressing or eliminating the rejection of a xenograft in a host which comprises administering to the host a pharmaceutical product or composition according to any one of Claims 1 to 9 containing an effective amount of a Class II major histocompatibility complex protein binding agent and, when present an effective amount of any other immunosuppressant or inhibitor of B-cell function.

Anti-hamster antibody titre from OX6γ1 treated and untreated xenografts.

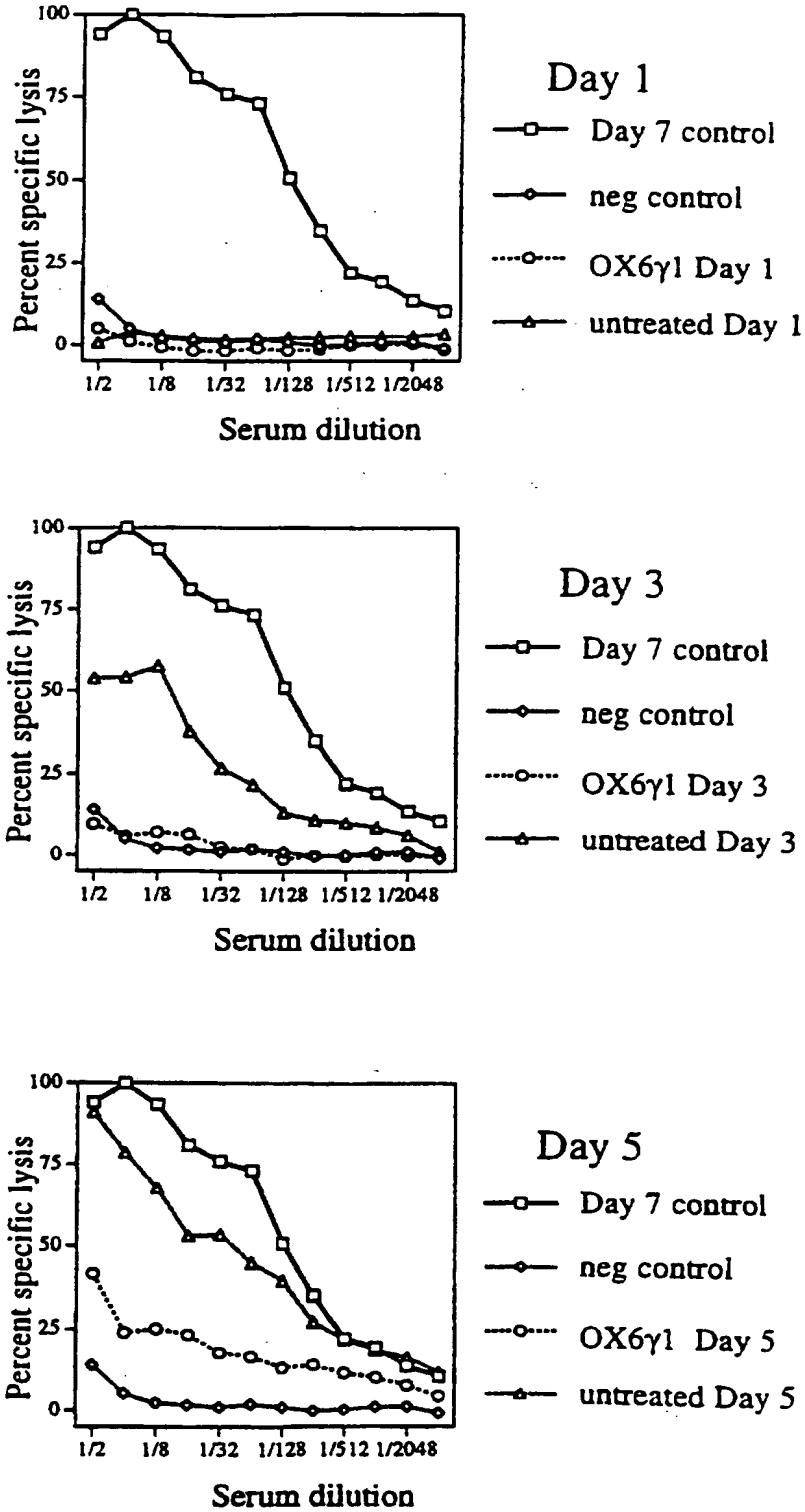

FIGURE 1

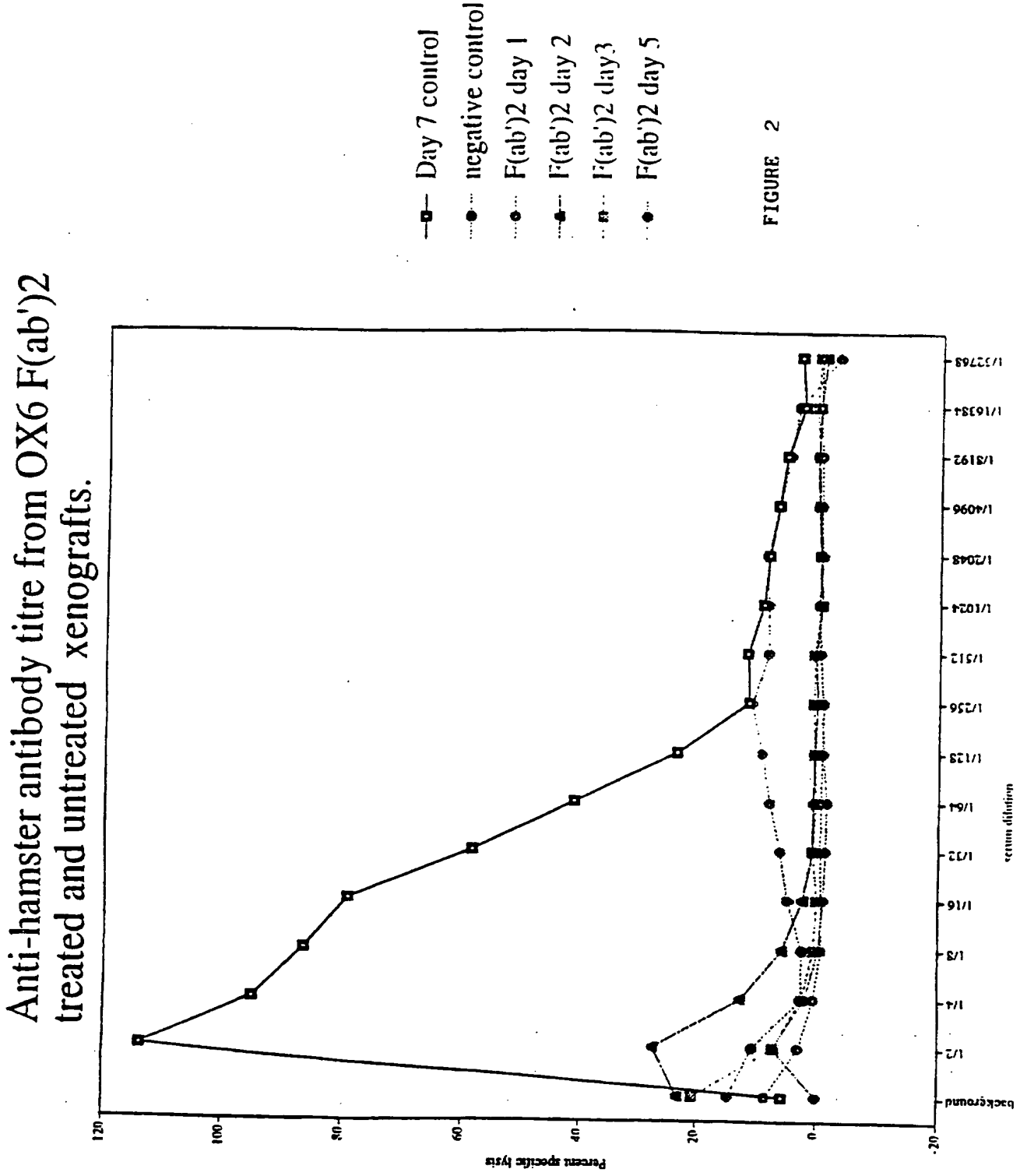

FIGURE 2

INTERNATIONAL SEARCH REPORT

Intern[illegible]al Application No
PCT[illegible]97/02599

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/395 A61K38/13 C07K16/28 C07K14/74

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 28897 A (US HEALTH) 22 December 1994 see page 8, line 14 - page 10, line 4	
A	PRUITT, SCOTT K. ET AL: "The effect of xenoreactive antibody and B cell depletion on hyperacute rejection of guinea pig-to-rat cardiac xenografts" TRANSPLANTATION (1993), 56(6), 1318-24 CODEN: TRPLAU;ISSN: 0041-1337, 1993, XP002051136 see pages 1320-1322, RESULTS	
P,A	WO 96 38543 A (DIACRIN INC) 5 December 1996	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 December 1997	16. 01. 98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Halle, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 97/02599

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 10

because they relate to subject matter not required to be searched by this Authority, namely:

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

Remark : Although claim 10 is directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

[redacted]ormation on patent family members

[redacted]el Application No
[redacted]/GB 97/02599

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9428897 A	22-12-94	AU 7056494 A	03-01-95
		CA 2164641 A	22-12-94
		EP 0702554 A	27-03-96
		JP 8511266 T	26-11-96
		US 5556754 A	17-09-96
WO 9638543 A	05-12-96	AU 5713696 A	18-12-96